

Figure 1

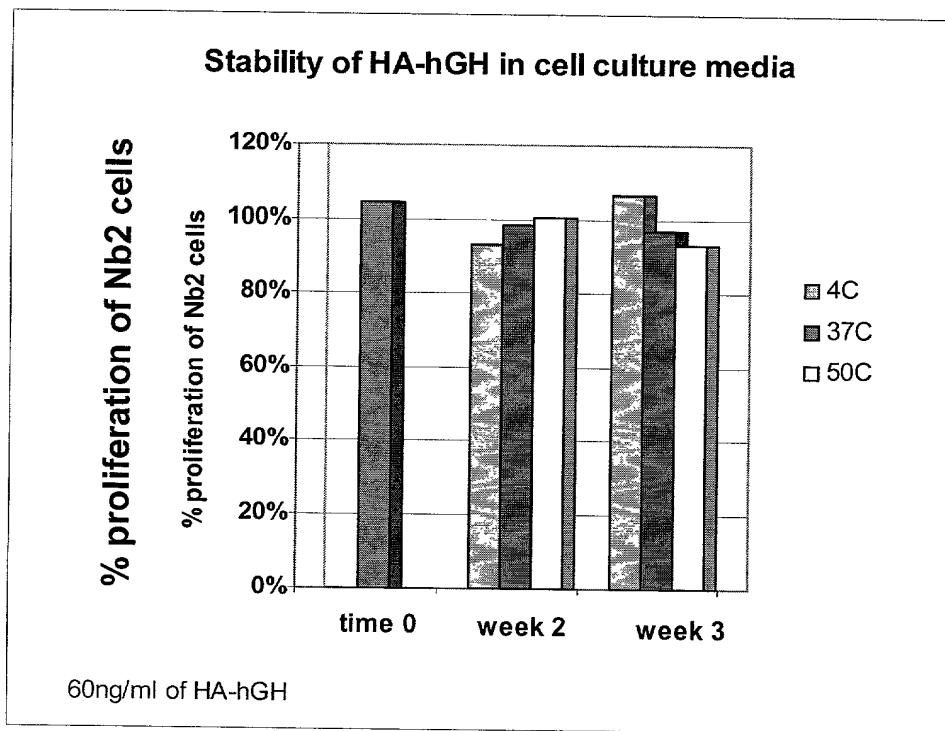


Figure 2

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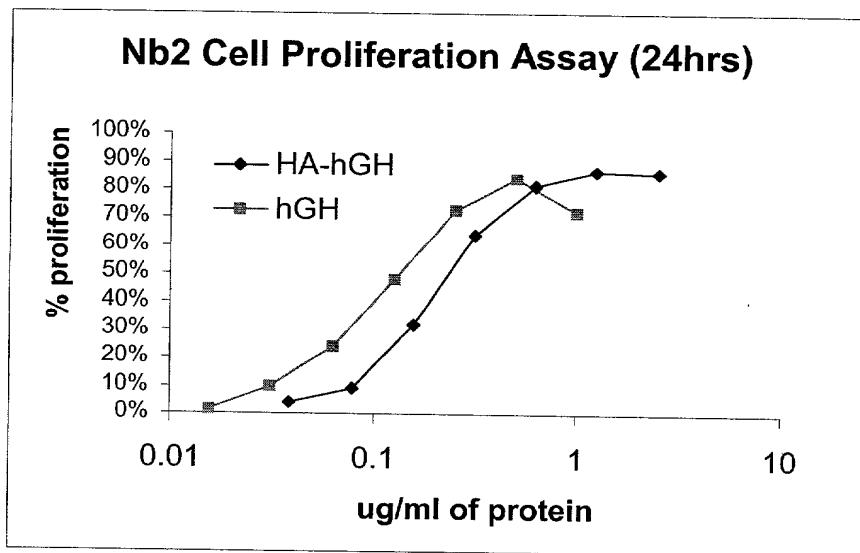


Figure 3A

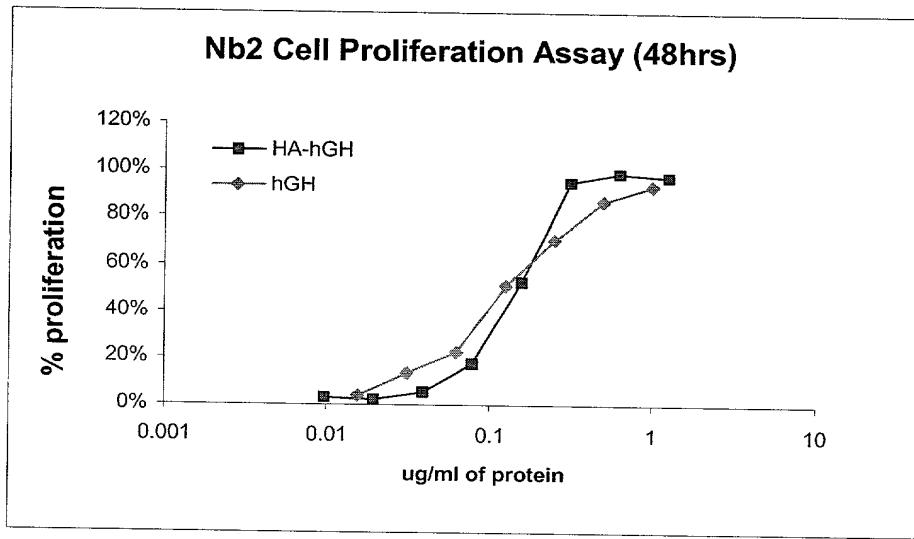


Figure 3B

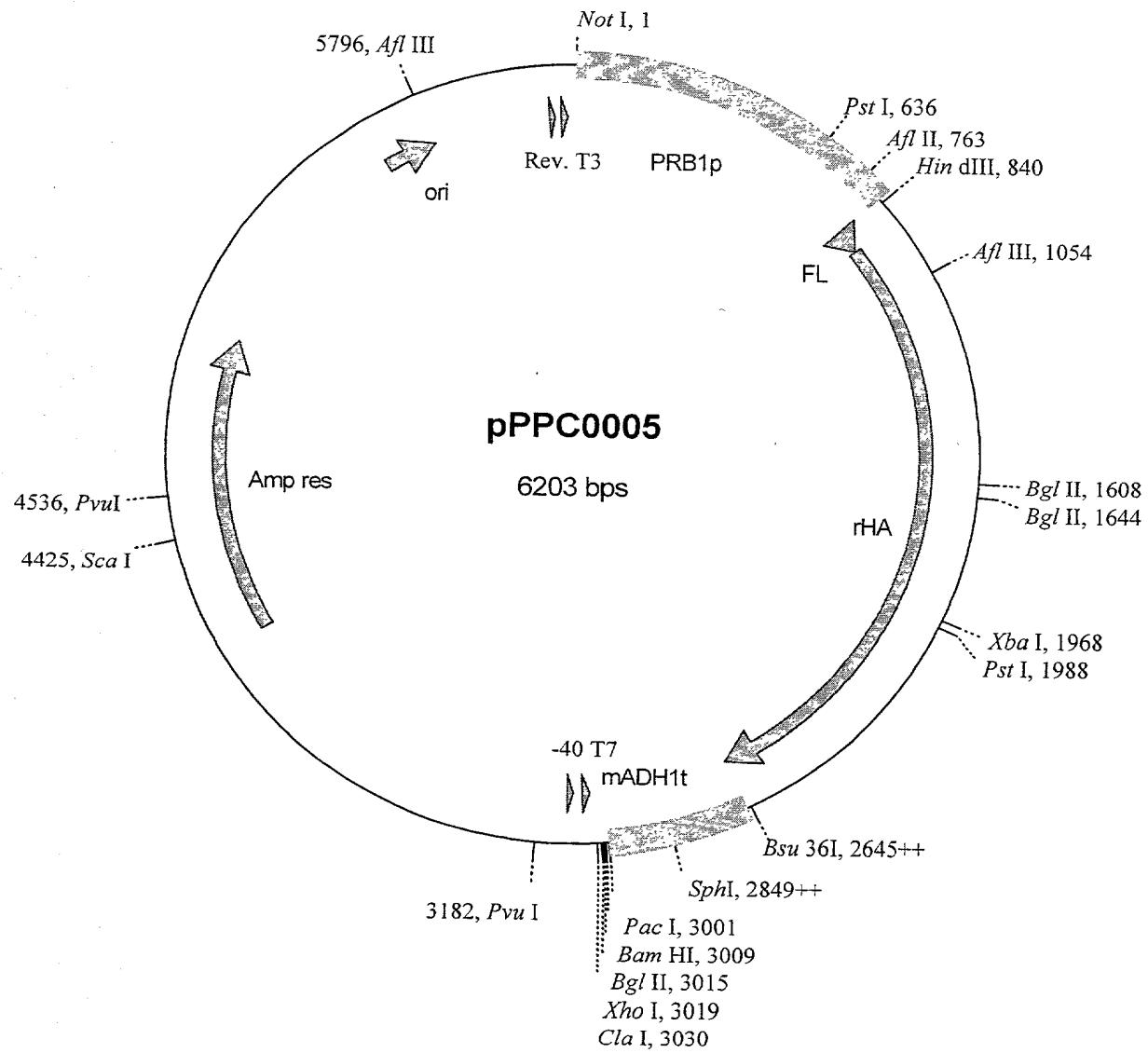


Figure 4

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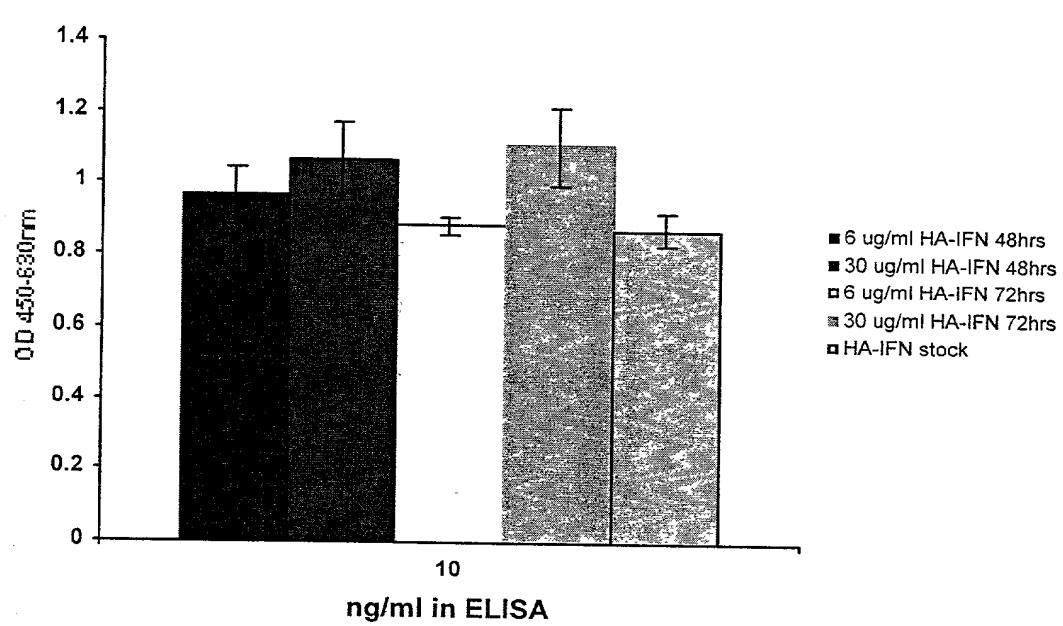
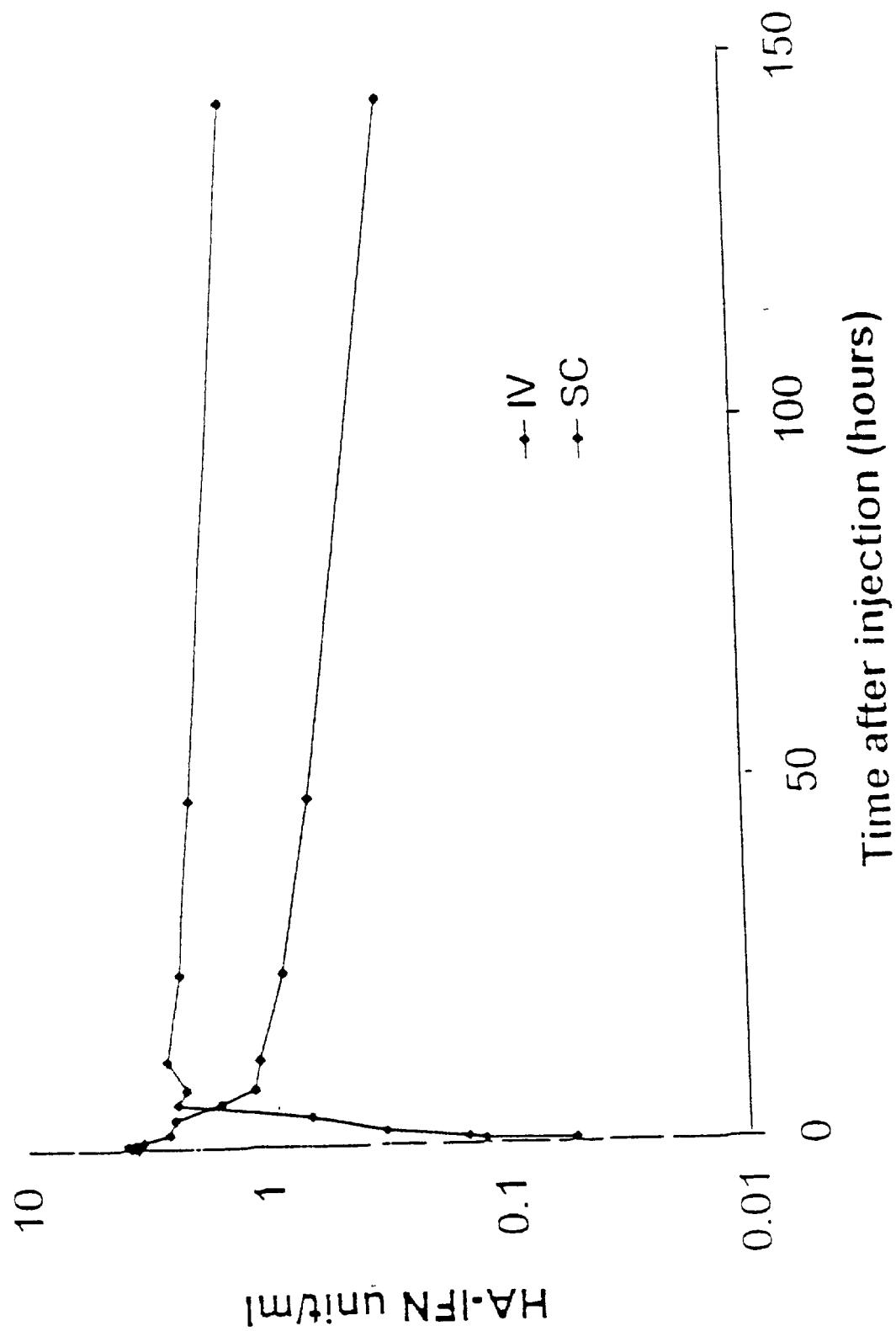


Figure 5

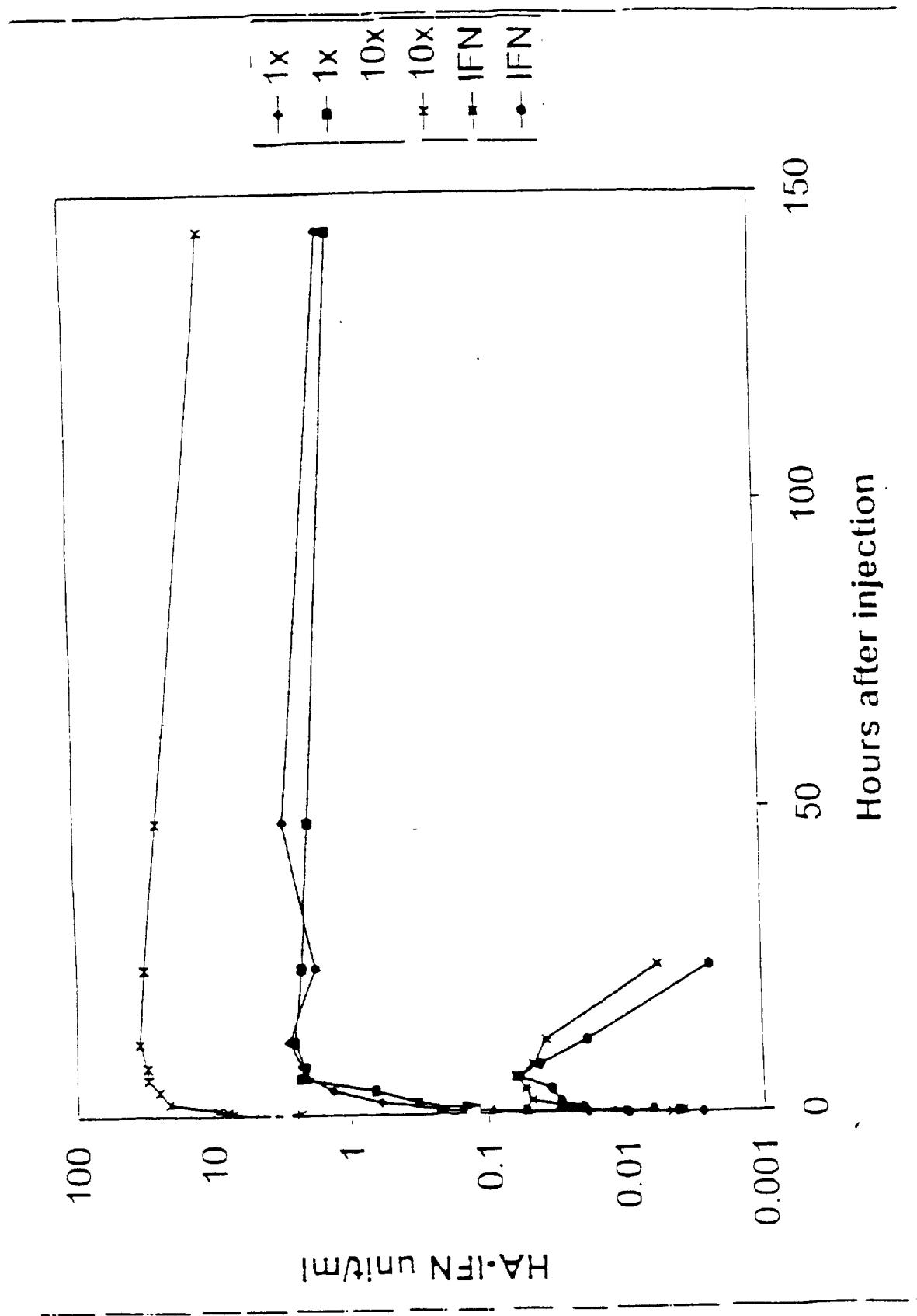
SCANNED, # 12

Figure 6



SCANNED, # 12

Figure 7



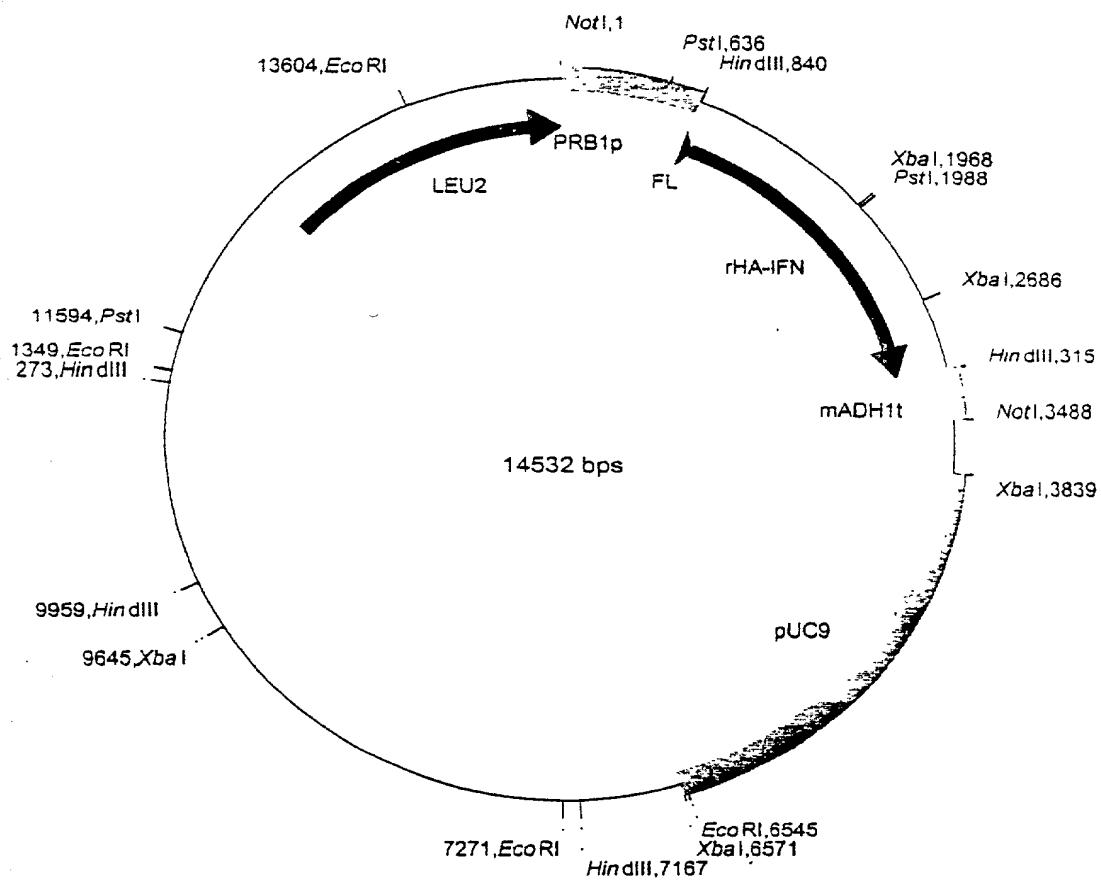


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.
HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III/Bam HI* fragment generally used.

Figure 8

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Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHH HHHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

IV

151 APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHH HHHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n

↓

IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHH HHHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

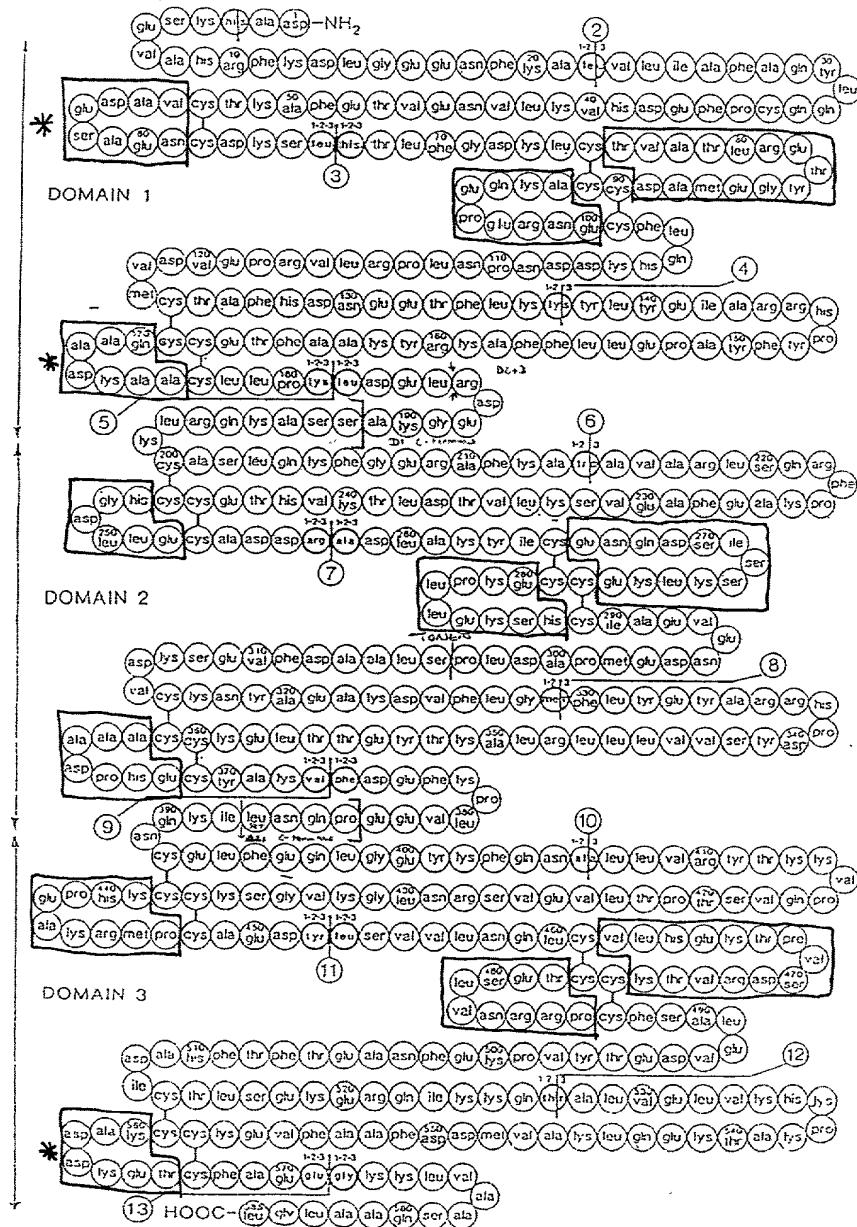


Figure 11

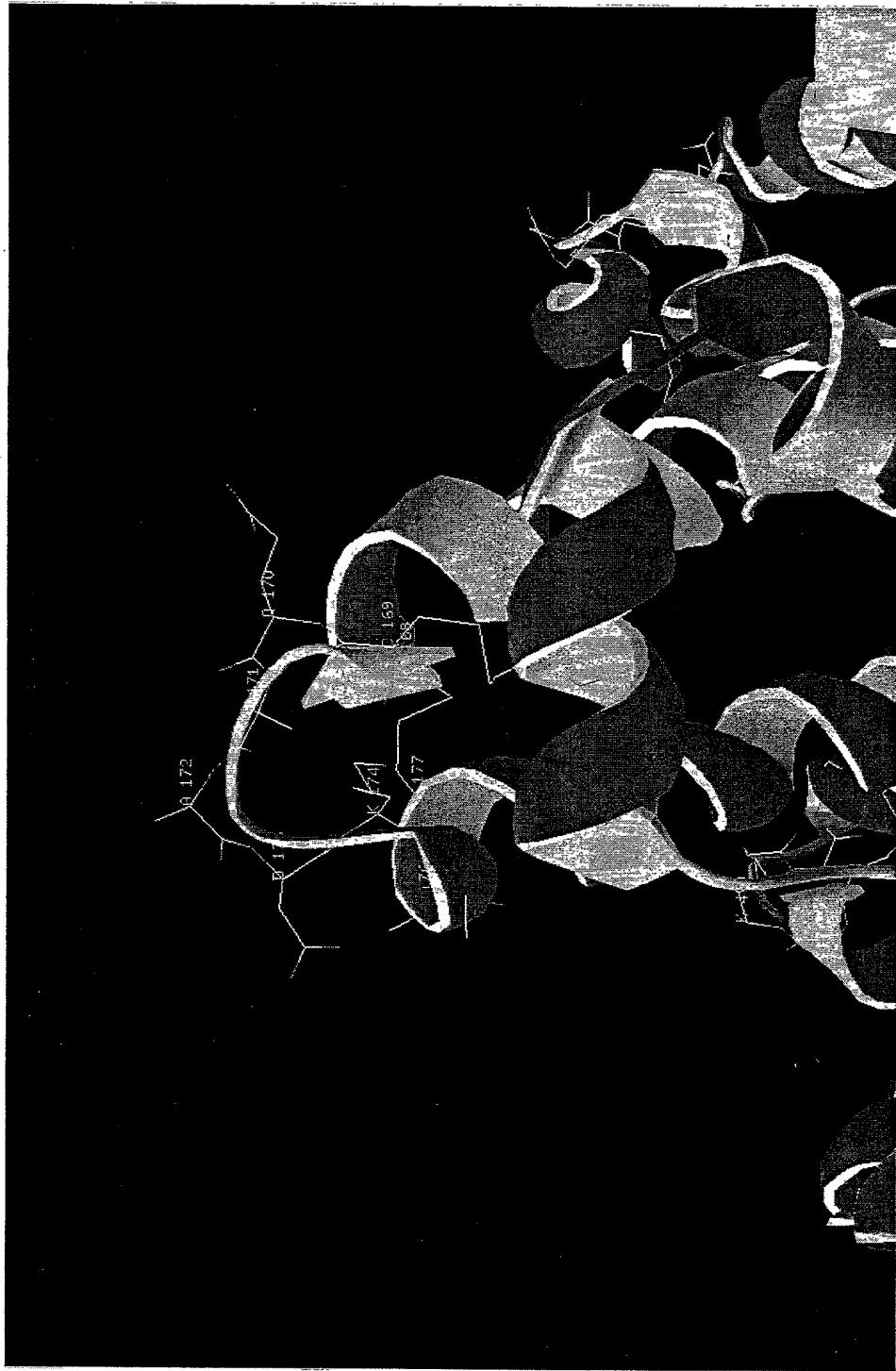


Figure 12: Loop IV Gln170-Ala176

Disulfide bonds shown in yellow

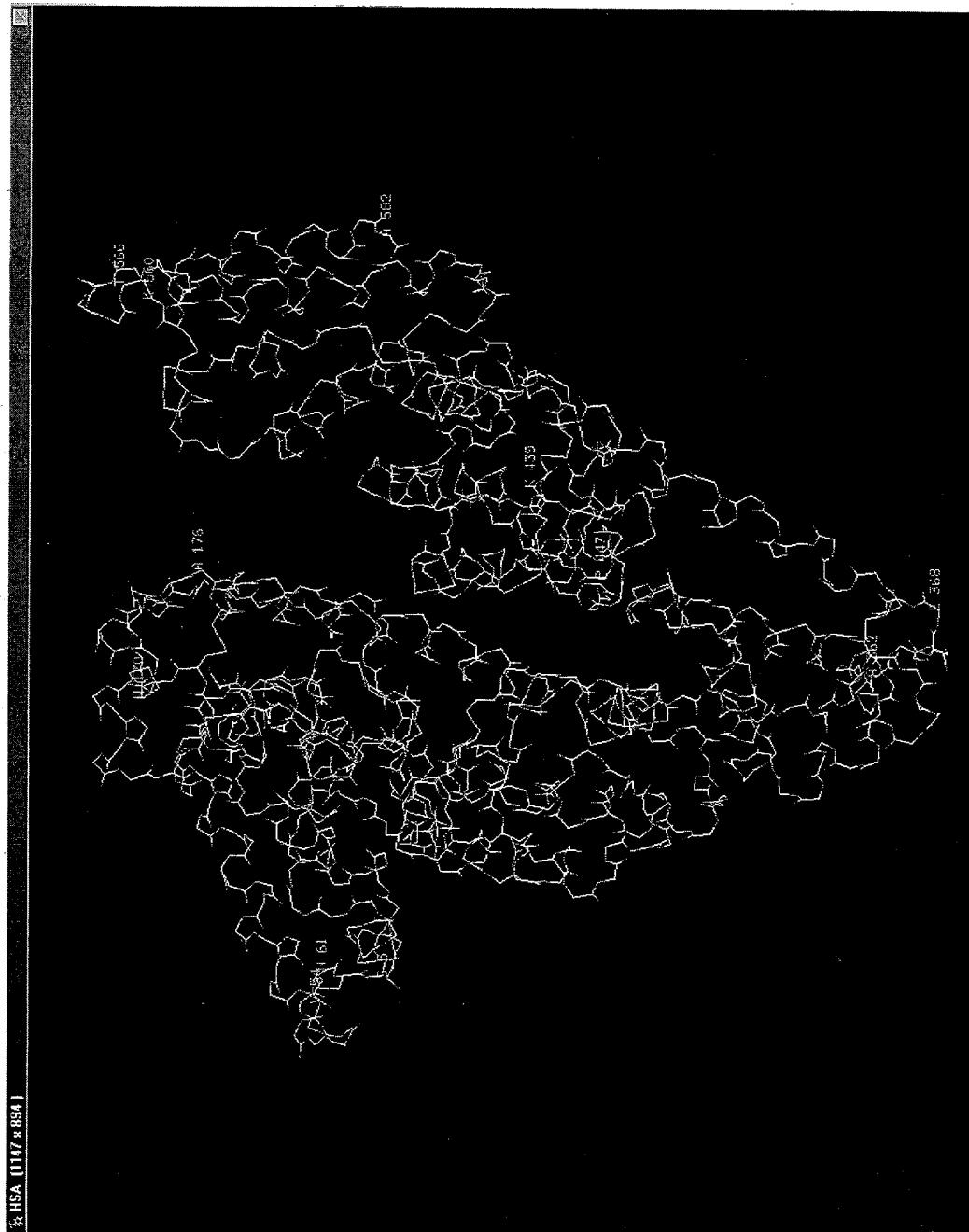
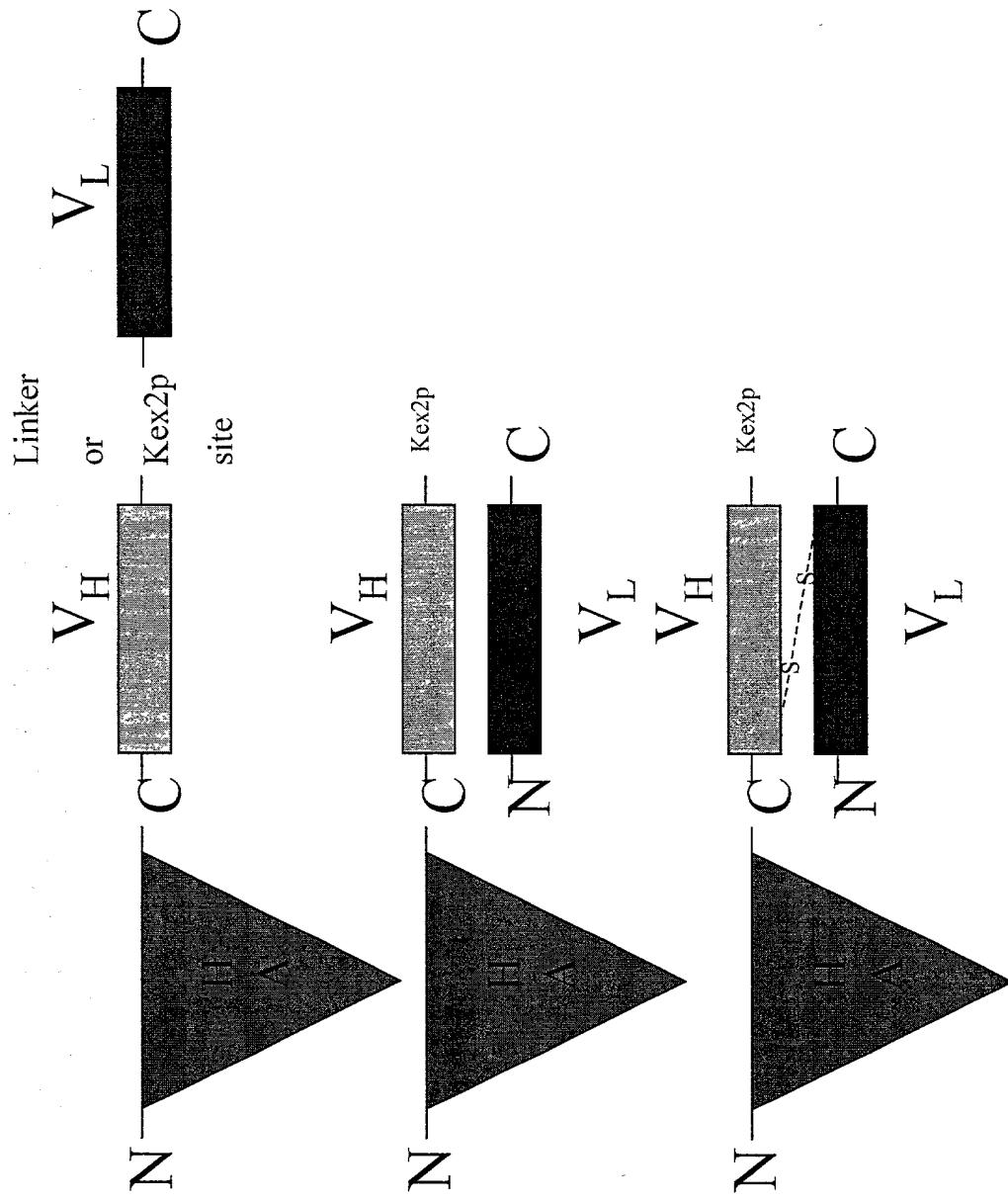


Figure 13: Tertiary Structure of HSA



**Figure 14: Schematic Diagram of Possible ScFv Fusions
(Example is of a C-terminal fusion to HA)**

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAC GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E I A R R H P Y F A P E L L F F A K K R 160

Figure 15A

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481	TAT	AAA	GCT	TTT	ACA	GAA	TGT	TGC	CAA	GCT	GCT	GAT	AAA	GCT	GCC	TGC	TGC	TTC	CCA	540	
161	Y	K	A	F	T	E	C	Q	A	D	K	A	A	C	L	L	P	P	180		
541	AAG	CTC	GAT	GAA	CTT	CGG	GAT	GAA	GGG	AAG	GCT	TCG	TCT	GCC	AAA	CAG	AGA	CTC	AAA	TGT	600
181	K	L	D	E	L	R	D	E	G	K	A	S	S	A	K	Q	R	L	K	C	200
601	GCC	AGT	CTC	CAA	AAA	TTT	GGA	GAA	AGA	GCT	TTC	AAA	GCA	TGG	GCA	GTC	GCG	CTG	AGC	660	
201	A	S	L	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	220
661	CAG	AGA	TTT	CCC	AAA	GCT	GAG	TTT	GCA	GAA	GTT	TCC	AAG	TTA	GTG	ACA	GAT	CTT	ACC	AAA	720
221	Q	R	F	P	K	A	E	F	A	E	V	S	K	L	V	T	D	L	T	K	240
721	GTC	CAC	ACG	GAA	TGC	CAT	GGA	GAT	CTG	CTT	GAA	TGT	GCT	GAT	GAC	AGG	GCG	GAC	CRT	780	
241	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	R	A	D	L	260	
781	GCC	AAG	TAT	ATC	TGT	GAA	AAT	CAG	GAT	TCG	ATC	TCC	AGT	AAA	CTG	AAG	GAA	TGC	TGT	GAA	840
261	A	K	Y	I	C	E	N	Q	D	S	I	S	S	K	L	K	E	C	C	E	280
841	AAA	CCT	CTG	TTG	GAA	AAA	TCC	CAC	TGC	ATT	GCC	GAA	GTG	GAA	AAT	GAT	GAG	ATG	CCT	GCT	900
281	K	P	L	L	E	K	S	H	C	I	A	E	V	E	N	D	E	M	P	A	300
901	GAC	TTG	CCT	TCA	TTA	GCT	GCT	GAT	TTT	GTT	GAA	AGT	AAG	GAT	GTT	TGC	AAA	AAC	TAT	GCT	960
301	D	L	P	S	L	A	A	D	F	V	E	S	K	D	V	C	K	N	Y	A	320

Figure 15B

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961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341 Y S V V L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTP CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

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Figure 15D